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Child-parent cascade screening for familial hypercholesterolemia in Slovenia: Insights from the pilot program

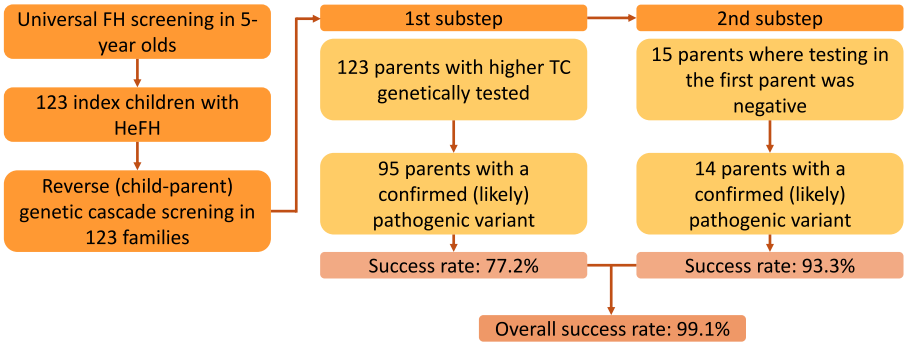
Jaka Šikonja [a,b], Kaja Kobale [a,c], Jan Kafol [a,c], Barbara Čugalj Kern [a,d], Matej Mlinarič [a,e], Ana Drole Torkar [a,e,f], Jernej Kovač [a,d], Matija Cevc [a,c], Zlatko Fras [a,c], Tadej Battelino [a,e], Urh Grošelj [a,e,f,*]

[a] Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000, Ljubljana, Slovenia
[b] Department of Endocrinology, Diabetes and Metabolic Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Zaloska 7, 1000, Ljubljana, Slovenia
[c] Department of Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Zaloska 7, 1000, Ljubljana, Slovenia
[d] Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Bohoričeva 20, 1000, Ljubljana, Slovenia
[e] Department of Endocrinology, Diabetes, and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Bohoričeva 20, 1000, Ljubljana, Slovenia
[f] Center for Rare Diseases. University Children's Hospital, University Medical Centre Ljubljana, Bohoričeva 20, 1000, Ljubljana, Slovenia

HIGHLIGHTS

- Child-parent genetic cascade screening following nationwide universal familial hypercholesterolemia screening is feasible.
- Child-parent cascade screening identified a parent with familial hypercholesterolemia in almost all families.
- Parents identified through cascade screening appear to have less established cardiovascular disease.

GRAPHICAL ABSTRACT



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ABSTRACT

Background and aims: Cascade familial hypercholesterolemia (FH) screening of parents could reduce the burden cardiovascular disease (CVD) in relatives of index cases by enabling timely diagnosis of FH. Here, we present the positive outcomes of the pilot child-parent cascade screening program in Slovenia.
Methods: One hundred and thirty-eight parents from 123 families of an index child with genetically confirmed FH were randomly included in the pilot child-parent cascade screening program. Index children were identified

* Corresponding author. Department of Endocrinology, Diabetes, and Metabolism and Center for Rare Diseases, University Children's Hospital Ljubljana, Bohoriceva 20, 1000, Ljubljana, Slovenia
E-mail addresses: jaka.sikonja@kclj.si (J. Šikonja), kaja.kobale@kclj.si (K. Kobale), jan.kafol@kclj.si (J. Kafol), barbara.kern@kclj.si (B. Čugalj Kern), matej.mlinaric@kclj.si (M. Mlinarič), ana.droletorkar@kclj.si (A. Drole Torkar), jernej.kovac@kclj.si (J. Kovač), matija.cevc@kclj.si (M. Cevc), zlatko.fras@kclj.si (Z. Fras), tadej.battelino@mf.uni-lj.si (T. Battelino), urh.groselj@kclj.si (U. Grošelj).

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through the universal FH screening program in preschool children. Genetic testing using Sanger sequencing was performed for cascade screening to detect (likely) pathogenic variants, previously confirmed in the index child. *Results:* The success rate of confirming a (likely) pathogenic variant was 77.2 % when the first parent, preferably with higher total cholesterol levels, was tested, and reached 99.1 % when the variant was identified in the first tested parent or when both parents were tested. In the minority of cases (13.8 %), parents had had a clinical diagnosis of FH prior to their child and these had somewhat higher prevalence of CVD compared to parents that were diagnosed after their index child through the pilot program (12.5 % vs. 4.3 %; $p = 0.382$).
Conclusions: In conclusion, the presented pilot child–parent cascade screening program is feasible in clinical practice and shows a high success rate in identifying parents with FH. Parents diagnosed through the program appeared to have a lower prevalence of CVD. However, larger cohorts are needed to confirm these findings.

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal semi-dominant disorder of lipoprotein metabolism, characterized by elevated low-density lipoprotein cholesterol (LDL-C) leading to accelerated atherosclerosis and increased risk for premature cardiovascular disease (CVD) [1]. FH is considered the most common non-modifiable CVD risk factor with an estimated prevalence of 1:313 [2] and is caused by pathological variants in the LDL receptor (*LDLR*), apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes [3].

Nearly 90 % of untreated individuals with FH will develop coronary artery disease, half of men and women before they turn 50 and 60 years of age, respectively [4]. Early identification of individuals with FH, combined with timely initiation of lipid-lowering therapy, significantly reduces the risk of CVD [5]. The greatest benefit is observed in those with the highest cholesterol levels and in whom treatment was started earlier in life [6].

Due to the inheritance pattern, every child with genetical FH has at least one parent and possibly also a sibling with FH, making cascade screening of relatives of index cases essential and viable for reducing the CVD burden in affected families. Adults with FH that were diagnosed through cascade screening tend to be younger and have less established CVD [7]. Prerequisite is the identification of index cases with FH which is done by universal screening and opportunistic testing in real world setting. Unfortunately, in Europe alone, it is estimated that less than 10 % of adults and 5 % of children are diagnosed, which prevents them from accessing timely and appropriate treatment [8,9]. Cascade screening alone is not sufficient to discover all individuals, thus a systematic combination of opportunistic testing, universal screening, and cascade screening strategies need to be employed for the highest success rate [10]. Moreover, the utilization of universal screening of children followed by reverse child-parent cascade screening was found to be cost effective compared to other approaches to FH identification [11].

Slovenia took the lead in this approach by successfully implementing a universal FH screening program for preschool children in 1995. Following the nationwide adoption of the program, a pilot child-parent cascade screening initiative was launched in 2012. In this article, we present the positive outcomes of this pilot which were instrumental in expanding the screening program, making child-parent cascade screening an integral part of standard clinical practice [12,13]. The three step screening program was approved by the Slovenian National Council of Pediatrics and by the National Health Council at the Ministry of Health.

2. Materials and methods

2.1. Study population and design

In this prospective cross-sectional study, we analyzed data from a pilot child-parent cascade FH screening program conducted in Slovenia between January 2012 and July 2021. Study was approved by the National Medical Ethics Committee of the Republic of Slovenia (n. 0120–286/2021/3).

The Slovenian screening program for FH includes three steps. Total cholesterol (TC) measurement is legally mandated in 5-year-old preschool children during regular health-checks with the primary care pediatrician [8]. This first step successfully reaches over 90 % of the approximately 18,000–20,000 eligible children in each generation, resulting in around 50 newly diagnosed children with FH each year. Children with elevated TC $>$ 6 mmol/L (240 mg/dL) or $>$ 5 mmol/L (190 mg/dL) with positive family history are referred, as part of the second step, to the pediatric outpatient lipid clinic at the University Children's Hospital Ljubljana where genetic testing is performed [14,15]. In cases where genetic testing identified a (likely) pathogenic variant in one of the three main genes associated with FH (*LDLR*, *APOB*, *PCSK9*) in an index child, pilot child-parent genetic cascade screening of the parents was conducted as part of the third step, preferably starting with the parent with higher TC levels (Fig. 1). If an FH-associated genetic variant was not identified in the firstly tested parent, cascade screening was performed in the other parent.

Parents were recruited randomly during regular outpatient visits of their children with FH. As this was a pilot program of cascade screening, it faced significant limitations, such as lack of funding for genetic testing and lack of staffing and administrative barriers to performing comprehensive out-patient evaluations of parents within the pediatric lipid clinic, resulting in a relatively low number of parents being included.

After obtaining written informed consent from parents for participation in the pilot program, a complete medical history was obtained, their electronic medical records were reviewed with an emphasis on personal history of hypercholesterolemia and CVD. CVD was defined as acute myocardial infarction, stroke, peripheral artery disease, angina pectoris, or a revascularization procedure prior to the time of the examination regardless of age at diagnosis or event. Venous blood was collected for the analysis of a comprehensive lipid panel and genetic testing. While some participants were taking lipid-lowering therapy at the time of blood collection, the reported lipid levels were not adjusted to reflect their lipid-lowering effect. Upon confirmation of a (likely) pathogenic variant in one of the three major FH genes, all parents who had not yet been under the care of a lipidologist were referred to a specialized adult lipid clinic.

TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, and triglycerides (TG) were measured using the Abbott Alinity C Chemistry Analyzer (Abbott Laboratories, USA). Lipoprotein(a) (Lp(a)), apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1) were determined through immunonephelometry using the Siemens Nephelometer Analyzer Atellica Neph 630 (Siemens Healthineers, Ireland).

Genetic testing was performed at the Institute for Special Laboratory Diagnostics at the University Children's Hospital, University Medical Centre Ljubljana. Genomic DNA was isolated from the whole blood sample according to the established laboratory protocols using FlexiGene isolation kit (Qiagen, Germany). While next-generation sequencing was used to identify disease-causing variants in children within the second step of the screening program, Sanger sequencing was used for confirmation of causative variants that were previously detected in their children. Genetic variants were interpreted according to the latest American College of Medical Genetics and Genomics (ACMG) guidelines [16]. Detailed genetic analysis procedure was already described in our previous publication [17].

Data was collected and analyzed using SPSS Statistics, version 26.0 (IBM, USA) and Microsoft Office Excel 365 (Microsoft Corporation, USA). Numerical variables were assessed for normality of distribution. Non-normally distributed variables were analyzed using Mann-Whitney test and presented as median (first quartile–third quartile; interquartile range [IQR]). Normally distributed variables were analyzed using independent samples *t*-test and presented as mean ± standard deviation. Categorical variables were analyzed with Pearson's chi-squared test and presented as absolute frequencies (relative frequencies in %). Two-sided statistical tests were used and a $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Child-parent cascade screening success rate

As part of the pilot child-parent cascade screening program, altogether 138 parents (68 [49.3 %] females, all Caucasians, median age 42.6 [IQR: 38.0–46.2] years) from 123 families underwent genetic testing and clinical assessment. In the first substep, 123 parents of index pediatric cases, predominantly with higher TC levels, were tested and (likely) pathogenic variants were found in 95 parents, implying of a success rate of 77.2 %. In 15 families where the initially tested parent was negative, the other parent was subsequently tested, and (likely) pathogenic variants were found in 14 of them, yielding a success rate of 93.3 % for this second substep. Together in families where the variant was found in the first tested parent and in families where both parents were tested, genetic cascade screening was successful in 109/110 (99.1 %) since the pathogenic variant was absent in both parents in only one family. Altogether, a likely pathogenic or pathogenic variant was confirmed in 109 tested parents (79.0 %), with both sexes being approximately equally represented (58 [53.2 %] female). The median age at examination of parents with genetically confirmed variants was comparable to the parents in whom the genetic testing was negative (42.0 [IQR: 38.0–46.2] years vs. 43.5 [IQR: 37.8–46.4] years; $p = 0.656$). Disease-causing variants were detected in the *LDLR* (79 [72.5 %]) and *APOB* (30 [27.5 %]) genes; however, no variants were identified in the *PCSK9* gene. This outcome reflects the genetic background of the pediatric index cases and the methods employed for genetic testing. The overview of detected variants in the included parents is presented in Table 1.

Table 1
Overview of detected likely pathogenic and pathogenic variants for FH in the pilot child-parent screening program. The variants are sorted by their frequency in our cohort. **Abbreviations:** ACMG-AMP – American College of Medical Genetics and Genomics – Association for Molecular Pathology; *APOB* – Apolipoprotein B gene; *LDLR* – Low-Density Lipoprotein Receptor gene.

Gene	Nucleotide change	Protein change	ACMG-AMP Classification	Frequency, N (%)
APOB	NM_000384.3: c.10580G > A	NP_000375.3:p. (Arg3527Gln)	Pathogenic	30 (27.5)
LDLR	NM_000527.5: c.1432G > A	NP_000518.1:p. (Gly478Arg)	Pathogenic	15 (13.8)
LDLR	NM_000527.5: c.557delG	NP_000518.1:p. (Gly186ValfsTer20)	Pathogenic	14 (12.8)
LDLR	NM_000527.5: c.858C > A	NP_000518.1:p. (Ser286Arg)	Pathogenic	8 (7.3)
LDLR	NM_000527.5: c.1754T > C	NP_000518.1:p. (Ile585Thr)	Pathogenic	7 (6.4)
LDLR	NM_000527.5: c.81C > G	NP_000518.1:p. (Cys27Trp)	Pathogenic	6 (5.5)
LDLR	NM_000527.5: c.2416dupG	NP_000518.1:p. (Val806GlyfsTer11)	Pathogenic	5 (4.6)
LDLR	NM_000527.5: c.1637G > A	NP_000518.1:p. (Gly546Asp)	Pathogenic	4 (3.7)
LDLR	NM_000527.5: c.1646G > A	NP_000518.1:p. (Gly549Asp)	Pathogenic	3 (2.8)
LDLR	NM_000527.5: c.1056C > G	NP_000518.1:p. (Cys352Trp)	Pathogenic	2 (1.8)
LDLR	NM_000527.5: c.1690A > G	NP_000518.1:p. (Asn564Asp)	Pathogenic	2 (1.8)
LDLR	NM_000527.5: c.1033C > T	NP_000518.1:p. (Gln345Ter)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.1151A > C	NP_000518.1:p. (Gln384Pro)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.1285G > A	NP_000518.1:p. (Val429Met)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.1567G > A	NP_000518.1:p. (Val523Met)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.1897C > T	NP_000518.1:p. (Arg633Cys)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.223T > A	NP_000518.1:p. (Cys75Ser)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.2389G > A	NP_000518.1:p. (Val797Met)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.347G > A	NP_000518.1:p. (Cys116Tyr)	Likely Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.415G > A	NP_000518.1:p. (Asp139Asn)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.662A > G	NP_000518.1:p. (Asp221Gly)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.1246C > T	NP_000518.1:p. (Arg416Trp)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.798T > A	NP_000518.1:p. (Asp266Glu)	Pathogenic	1 (0.9)
LDLR	NM_000527.5: c.862G > A	NP_000518.1:p. (Glu288Lys)	Pathogenic	1 (0.9)

3.2. Clinical characteristics

At the date of examination, in the whole cohort average TC was 6.6 ± 1.6 mmol/L [255.2 ± 62 mg/dL], LDL-C 4.5 ± 1.5 mmol/L [174 ± 58 mg/dL], non-HDL-C 5.1 ± 1.5 mmol/L [197 ± 58 mg/dL], APOB 1.26 ± 0.31 g/L and median Lp(a) 143.5 (<99.4–549) mg/L. Parents with a confirmed FH–causing variant (carriers) showed significantly more adverse lipid profiles compared to non-carriers (Table 2), whereas the lipid profiles were not adjusted for the effect of the lipid-lowering therapy. Carriers had notably higher levels of TC (6.8 ± 1.6 vs. 5.6 ± 0.9 mmol/L; $p < 0.001$), LDL-C (4.8 ± 1.5 vs. 3.6 ± 0.8 mmol/L; $p < 0.001$), non-HDL-C (5.3 ± 1.6 vs. 4.2 ± 0.9 mmol/L; $p < 0.001$), and ApoB (1.30 ± 0.31 vs. 1.08 ± 0.20 g/L; $p = 0.001$), as well as higher historical maximum values for TC and LDL-C ($p < 0.001$ for both comparisons). TG levels were slightly lower in carriers compared to non-

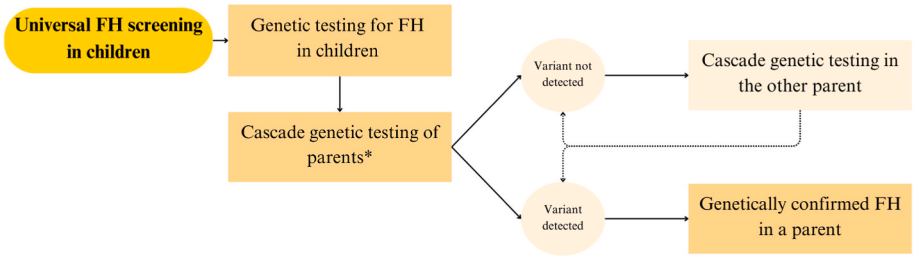

Fig. 1. Pilot child-parent cascade screening program for familial hypercholesterolemia in Slovenia. * Preferably, parents with higher total cholesterol levels were tested.

Table 2
Comparison of lipid profiles between parents with a confirmed likely pathogenic or pathogenic variant (carriers) and those without an identified variant (non-carriers). Data are mean ± standard deviation and median (first quartile–third quartile). Independent samples *t*-test and Mann-Whitney Test were used for comparison between FH variant carriers and non-carriers. **Abbreviations:** TC – total cholesterol; HDL-C – High-Density Lipoprotein Cholesterol; LDL-C – Low-Density Lipoprotein Cholesterol; TG – Triglycerides; Lp(a) – Lipoprotein(a); ApoA1 – Apolipoprotein A1; ApoB – Apolipoprotein B. Max TC and LDL-C – maximal recorded TC in the available health records.

	Carriers (N = 109)	Non-carriers (N = 29)	*p*
Female, N (%)	58 (53.2)	10 (34.5)	0.073
Age, year	42.0 (38.0–46.2)	43.5 (37.8–46.4)	0.656
TC, mmol/L	6.8 ± 1.6	5.6 ± 0.9	**<0.001**
Non-HDL-C, mmol/L	5.3 ± 1.6	4.2 ± 0.9	**<0.001**
HDL-C, mmol/L	1.5 ± 0.4	1.4 ± 0.5	0.365
LDL-C, mmol/L	4.8 ± 1.5	3.6 ± 0.8	**<0.001**
TG, mmol/L	1.2 ± 0.7	1.5 ± 0.8	0.109
Lp(a), mg/L	145.5 (<99.9–549)	131 (<99.4–505)	0.854
ApoA1, g/L	1.50 ± 0.25	1.51 ± 0.29	0.900
ApoB, g/L	1.30 ± 0.31	1.08 ± 0.20	**0.001**
Max TC, mmol/L	7.5 ± 2.2	5.6 ± 0.9	**<0.001**
Max LDL-C, mmol/L	5.5 ± 2.1	3.6 ± 0.8	**<0.001**

carriers (1.2 ± 0.7 vs. 1.5 ± 0.8), however this was not statistically significant ($p = 0.109$). No significant differences were observed in HDL-C, Lp(a), or ApoA1 levels.

In the overall cohort, 3/64 (4.7 %) had a personal history of CVD, including acute myocardial infarction, stroke, peripheral artery disease, angina pectoris, or a revascularization procedure prior to the time of the examination. This was primarily driven by parents with a confirmed (likely) pathogenic variant (3/55; 5.5 %), whereas none of the parents without an identified variant had established CVD. FH-variant carriers with established CVD were generally older compared to those without CVD (median age 54.1 [range 44.0–54.1] vs. 43.3 [IQR: 38.1–46.4] years; $p = 0.054$), however the difference was borderline statistically significant.

3.3. Diagnosis and management at lipid specialist

The median age of parents with a (likely) pathogenic variant at the time their 5-year-old index child was screened was 35.2 (IQR: 31.5–38.8) years. In the pilot program, the median time from the child's screening to the parent's genetic FH diagnosis was 6.8 (IQR: 4.1–9.7) years, with a corresponding mean parental age of 42.1 (IQR: 38.3–46.7) years at diagnosis. The diagnosis of FH was first made in an index child in 94/109 [86.2 %] of cases, whereas the remaining 15 [13.8 %] parents with a diagnosis of FH prior to their child were diagnosed clinically using Dutch Lipid Clinic Network criteria. Parents who had had FH diagnosed prior to their children were of comparable ages to parents who were diagnosed through the pilot program (median age at genetic FH diagnosis 42.1 [IQR: 38.3–46.4] vs. 39.6 (IQR: 35.7–45.9); $p = 0.254$). There was a trend that parents who were diagnosed prior to the index child had a personal history of CVD in 1/8 (12.5 %) compared to 2/47 (4.3 %) where the child was first diagnosed, however this was not statistically significant due to low absolute frequencies established CVD in our cohort ($p = 0.382$, Fisher's Exact Test). Moreover, the majority of parents with FH (62 [56.9 %]) were not managed at a lipid specialist prior to the inclusion into the pilot screening program.

4. Discussion

Cascade screening of relatives of individuals with FH aids the identification of others with the same condition, liking them to appropriate care to reduce their CVD risk. The goal is to diagnose the individual in the subclinical phase when an CVD event has not yet occurred [18]. The combination of a universal FH screening of children and a child-parent genetic screening program has the potential to address these assumptions. In this article, we presented the positive outcomes of a pilot child-parent cascade screening program in Slovenia, which was instrumental to the expansion of the FH screening program in the country.

Following the universal FH screening program in preschool children, child-parent cascade screening of index cases with genetically confirmed FH achieved a high success rate (77.2 %) when the first parent, preferably with higher TC levels, was tested. Moreover, the strategy identified a parent with FH in nearly all families (99.1 %) where both parents were tested or where the variant was identified in the first tested parent. Cascade screening based on genetic testing was already found to be feasible and effective in several countries, including the Netherlands, Australia, Scotland and many others [19–21]. Child-parent cascade screening is estimated to identify one parent with FH per every index child, in the context that both parents are genetically tested, and even more if further cascade screening is performed from affected parents [22,23]. Our approach, in which the parent with the higher TC is tested first, successfully identifies a pathogenic variant in nearly every family while reducing costs and workload, as genetic testing of both parents is commonly not required.

A pathogenic variant was not identified in only one family, which may be attributed either to non-paternity or to a *de novo* variant, however, FH caused by a *de novo* variant is very rare and has been reported in a limited series of cases in the literature [24]. Non-paternity findings arising from genetic testing conducted for purposes other than determining biological parentage present a significant ethical and moral dilemma. There is no clear professional consensus on how such results should be disclosed; however, it is widely recognized that each case is unique, and clinicians must adopt tailored approaches in communicating these findings to the family, particularly to the child. In this context, counseling and pre-test consent are crucial in preparing families for the possibility of non-paternity results, with genetic counselors providing essential guidance and support throughout the process [25].

The overall prevalence of CVD in the cohort of parents diagnosed with FH was low (around 6 %), which reflects their age, as most were diagnosed in their forties (median age 42.1 [38.3–46.7] years). However, due to the nature of the pilot program there was a considerable delay of around 6.8 years between the screening of the child and establishment of diagnosis in the parent. This potential caveat could be minimized through an established cascade screening program with clear protocols for genetic testing in parents to minimize the time to diagnosis. A minority of parents with FH had had an established clinical FH prior to the cascade screening, however, we observed a trend that these parents were more likely to have CVD at screening compared to parents that were diagnosed through the pilot program (12.5 % vs. 4.3 %), implying that earlier diagnosis through cascade screening could reduce their risk of developing CVD or the fact that FH was diagnosed opportunistically after a CVD event [26].

Previous analyses of the management and treatment of individuals with FH have demonstrated their substantial benefit in terms of cost-effectiveness [10]. However, most of these analyses are based on theoretical calculations or on established cascade screening programs worldwide where adults represent the majority of index cases. Universal screening for LDL-C in 1–2-year-old children in the United Kingdom, followed by molecular genetic testing and reverse (child–parent) cascade screening, was found to be cost-effective, resulting in incremental cost-effectiveness ratio (ICER) of £12,480/quality-adjusted life year (QALY) [11]. Moreover, in Kagawa, Japan, universal screening of 9–10-year-old children and reverse cascade screening of their adult relatives was proven to be cost-effective compared to no screening with ICER of USD 1042/QALY [27]. Both ICERs were well below the respective countries' willingness-to-pay thresholds. These studies represents the highest level of evidence for the cost-effectiveness of FH screening programs similar to one in Slovenia, although a country-specific analysis for Slovenia has not yet been conducted.

Although clear professional guidelines exist for the consistent

implementation of cascade screening among relatives of index cases with FH, the overall uptake of screening in routine clinical practice remains limited. The reasons behind effective and widespread implementation of cascade screening for FH are multifaceted, encompassing systemic organization of the healthcare system, funding, legal frameworks, and barriers at the individual level among those involved in the program. Nevertheless, experiences from countries with high uptake, such as the Netherlands and Norway, suggest that public funding and the establishment of a centralized administrative body are essential to ensure systematic program implementation. Moreover, legal restrictions pose perhaps an even greater challenge, particularly regarding the option of healthcare professionals to contact relatives directly due to limitations in disclosing sensitive health information [28]. At the individual level, major barriers include lack of awareness of FH among both the general public and healthcare professionals, concerns about disease-related stigma, and insufficient educational materials. On the other hand, positive factors include good accessibility to specialized centers for ongoing patient management and strong awareness of FH among affected individuals [29]. While data on the topic were not collected systematically, we estimate that during the implementation of the pilot program, parents demonstrated good willingness to participate in the cascade screening initiative, and their responses were generally positive. However, achieving this required a considerable additional investment of time in raising awareness and educating parents. The main barriers identified from a standpoint of pediatric lipidologist were the limited time available and competing priorities during outpatient visits, as well as the absence of a dedicated coordination center responsible for program management, scheduling of appointments, and communicating results to the parents involved.

Important limitations of our article include the low absolute number of participants, the lack of clinical data on the use of lipid-lowering therapy, baseline untreated lipid values, a significant diagnostic delay between the times of diagnosis in the child and their parent, and the low absolute prevalence of CVD in the cohort. One of the main reasons for the mentioned limitations is the pilot design of the cascade program itself, which was carried out outside of routine clinical practice. The main barriers were the lack of financing of outpatient visits for parents, genetic testing, and additional administrative obstacles, which resulted in only a limited number of individuals included and low CVD prevalence. Thus, larger clinical studies are needed to validate the findings of this pilot program. We expect that the implementation of cascade testing will be significantly strengthened with the upgrade of the screening program, once it becomes part of routine clinical practice with secured administrative, human, and financial resources. Moreover, data on lipid-lowering therapy were not routinely collected; therefore, it was not possible to adjust lipid parameters according to the type and potency of therapy. To address this limitation, we reported the highest measured values of TC and LDL-C in parents, as these are generally a more accurate reflection of the genetic background of hyperlipidemia.

In conclusion, the presented pilot child–parent cascade screening program is feasible in clinical practice and shows a high success rate in identifying parents with FH. Parents diagnosed through the program appeared to have a lower prevalence of CVD, however, larger cohorts are needed to confirm our observations.

CRediT author statement

JŠ;: Investigation; Data Curation, Formal Analysis; Writing - Original Draft; **KK**: Investigation, Visualization, Writing - Original Draft; **JK**: Investigation, Writing - Original Draft; **BČK**: Investigation, Writing - Original Draft; **MM**: Investigation, Writing - Original Draft; **ADT**: Investigation, Writing - Original Draft; **JK**: Methodology, Validation, Investigation, Writing - Review & Editing; **MC**: Validation, Investigation, Writing - Review & Editing; **ZF**: Validation, Investigation, Writing - Review & Editing; **TB**: Supervision, Conceptualization, Investigation, Writing - Review & Editing; **UG**: Conceptualization, Validation, Investigation, Writing - Review & Editing.

Ethics considerations

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (n. 0120–286/2021/3). The study was conducted in accordance with the Declaration of Helsinki. Informed consent for genetic analysis and publication of anonymized data, was obtained from each participant.

Originality of content

We confirm all information and materials in the manuscript are original.

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Declaration of competing interest

Authors declare no conflict of interest.

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We sincerely thank all parents and their families who participated in this study and Urša Šuštar, PhD for her valuable contribution to the genetic analyses. U.G. had full access to all study data and takes responsibility for its integrity and accuracy. During the preparation of this work the author(s) used ChatGPT in order to improve the clarity and readability of the language used. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data availability

Data is available from the corresponding author upon reasonable request.

References

[1] Risk of fatal coronary heart disease in familial hypercholesteSci. Steering Committee Simon Broome Register Group. BMJrolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group BMJ 1991 Oct 12;303 (6807):893–6.
[2] Beheshti SO, Madsen CM, Varbo A, Nordestgaard BG. Worldwide prevalence of familial hypercholesterolemia. J Am Coll Cardiol 2020 May;75(20):2553–66.
[3] Hegele RA, Borén J, Ginsberg HN, Arca M, Averna M, Binder CJ, et al. Rare dyslipidaemias, from phenotype to genotype to management: a European Atherosclerosis Society task force consensus statement. Lancet Diabetes Endocrinol 2020 Jan;8(1):50–67.
[4] Gidding SS. Why cholesterol testing in children and adolescents matters. JAMA Cardiol 2016 Nov 1;1(8):859–61.
[5] Luirink IK, Wiegman A, Kusters DM, Hof MH, Groothoff JW, de Groot E, et al. 20-Year Follow-up of statins in children with familial hypercholesterolemia. N Engl J Med 2019 Oct 17;381(16):1547–56.
[6] Robinson JG, Williams KJ, Gidding S, Borén J, Tabas I, Fisher EA, et al. Eradicating the burden of atherosclerotic cardiovascular disease by lowering apolipoprotein B lipoproteins earlier in life. JAHA 2018 Oct 16;7(20).
[7] Vallejo-Vaz AJ, Stevens CAT, Lyons ARM, Dharmayat KI, Freiberger T, Hovingh GK, et al. Global perspective of familial hypercholesterolaemia: a cross-sectional study from the EAS familial Hypercholesterolaemia Studies Collaboration (FHSC). Lancet 2021 Sep:S0140673621011223.
[8] Kafol J, Miranda B, Sikonja R, Sikonja J, Wiegman A, Medeiros AM, et al. Proposal of a familial hypercholesterolemia pediatric diagnostic Score (FH-PeDS). Eur J Prev Cardiol 2025 Jun 20:zwaf352.

[9] Vallejo-Vaz AJ, De Marco M, Stevens CAT, Akram A, Freiberger T, Hovingh GK, et al. Overview of the current status of familial hypercholesterolaemia care in over 60 countries - the EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). Atherosclerosis 2018 Oct;277:234–55.
[10] Gidding SS, Wiegman A, Groselj U, Freiberger T, Peretti N, Dharmayat KI, et al. Paediatric familial hypercholesterolaemia screening in Europe: public policy background and recommendations. Eur J Prev Cardiol 2022 Sep 5:zwac200.
[11] McKay AJ, Hogan H, Humphries SE, Marks D, Ray KK, Miners A. Universal screening at age 1–2 years as an adjunct to cascade testing for familial hypercholesterolaemia in the UK: a cost-utility analysis. Atherosclerosis 2018 Aug; 275:434–43.
[12] Klančar G, Grošelj U, Kovač J, Bratanič N, Bratina N, Trebušak Podkrajšek K, et al. Universal screening for familial hypercholesterolemia in children. J Am Coll Cardiol 2015 Sep 15;66(11):1250–7.
[13] Groselj U, Wiegman A, Gidding SS. Screening in children for familial hypercholesterolaemia: start now. Eur Heart J 2022 May;4:ehac224.
[14] Groselj U, Kovac J, Sustar U, Mlinaric M, Fras Z, Podkrajsek KT, et al. Universal screening for familial hypercholesterolemia in children: the Slovenian model and literature review. Atherosclerosis 2018 Oct;277:383–91.
[15] Groselj U, Kafol J, Molk N, Sedej K, Mlinaric M, Sikonja J, et al. Prevalence, genetic variants, and clinical implications of hypocholesterolemia in children. Atherosclerosis 2025 Jan;400:119065.
[16] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015 May;17(5):405–24.
[17] Sustar U, Kordonouri O, Mlinaric M, Kovac J, Arens S, Sedej K, et al. Universal screening for familial hypercholesterolemia in 2 populations. Genet Med 2022 Oct 1;24(10):2103–11.
[18] Knowles JW, Rader DJ, Khoury MJ. Cascade screening for familial hypercholesterolemia and the use of genetic testing. JAMA 2017 Jul 25;318(4): 381.
[19] Huijgen R, Kindt I, Verhoeven SBJ, Sijbrands EJG, Vissers MN, Kastelein JJP, et al. Two years after molecular diagnosis of familial hypercholesterolemia: majority on cholesterol-lowering treatment but a minority reaches treatment goalReitsma PH, editor. PLoS One 2010 Feb 15;5(2):e9220.
[20] Finnie RM. Cascade screening for familial hypercholesterolaemia in Scotland. Diabetes Vascular Disease 2010 May;10(3):123–5.
[21] Hooper AJ, Nguyen LT, Burnett JR, Bates TR, Bell DA, Redgrave TG, et al. Genetic analysis of familial hypercholesterolaemia in Western Australia. Atherosclerosis 2012 Oct;224(2):430–4.
[22] Martin AC, Hooper AJ, Norman R, Nguyen LT, Burnett JR, Bell DA, Brett T, Garton-Smith J, Pang J, Nowak KJ, Watts GF. Pilot study of universal screening of children and child-parent cascade testing for familial hypercholesterolaemia in Australia. J Paediatr Child Health 2022 Feb;58(2):281–7.
[23] Wald DS, Wald NJ. Integration of child–parent screening and cascade testing for familial hypercholesterolaemia. J Med Screen 2019 Jun;26(2):71–5.
[24] Tada H, Hosomichi K, Okada H, Kawashiri M, Nohara A, Inazu A, et al. A de novo mutation of the LDL receptor gene as the cause of familial hypercholesterolemia identified using whole exome sequencing. Clin Chim Acta 2016 Jan 30;453:194–6.
[25] Cunningham E, Hays S, Wainstein T, Zierhut H, Virani A, Tryon R. Exploring genetic counselors' experiences with non-paternity in clinical settings. J Genet Counsel 2025 Feb;34(1):e1881.
[26] Gidding SS, Blom DJ, McCrindle B, Ramaswami U, Santos RD, Watts GF, et al. Life course approach for managing familial hypercholesterolemia. JAHA 2025 Mar 21: e038458.
[27] Matsunaga K, Harada-Shiba M, Yamashita S, Tada H, Uda A, Mori K, et al. A cost-effectiveness analysis for the combination of universal screening at 9-10 years old and reverse Cascade screening of relatives for familial hypercholesterolemia in Japan. J Anal Toxicol 2025 Aug 1;32(8):962–81.
[28] Andersen R, Andersen L. Examining barriers to cascade screening for familial hypercholesterolemia in the United States. J Clinical Lipidol 2016 Mar;10(2): 225–7.
[29] Dickson MA, Zahavich L, Rush J, Hewson S, Chitayat D, McCrindle BW, et al. Exploring barriers and facilitators to indirect Cascade screening for familial hypercholesteraemia in a Paediatric/Parent population. CJC Pediatric Congenital Heart Dis 2023 Oct;2(5):211–8.